Determination of acute toxicity of sodium pyrithione on common carp and its effects on some hormones and hematological parameters

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Abstract

In this study, the LC_{50}-24 h value of sodium pyrithione (NaPT) on *Cyprinus carpio* was determined 102.7643 µg/L. The effects of NaPT exposure on hematological parameters and some hormones in carp were also studied. In the groups exposed to NaPT compared to the control group, there was a decrease in the erythrocyte count (RBC), hemoglobin (Hb), hematocrit (Hct) values, total leukocyte count (WBC), lymphocyte and monocyte ratios, while an increased ratio was observed in granulocyte. Hormone analysis revealed a decrease in serum growth hormone (GH), insulin-like growth factor-1 (IGF-1), Thyroid-stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroxine (FT4) levels, while an increase was observed in total triiodothyronine (TT3), total thyroxine (TT4), adrenocorticotropic hormone (ACTH) and cortisol levels. Depending on the duration of exposure, a decrease was observed in the ratios of RBC, Hb, Hct, WBC, lymphocytes, monocytes, GH, IGF-1, TSH, TT3, and TT4 in both dose groups, while a decrease was observed in FT3 levels in the NaPT-20 dose group. ACTH and cortisol levels were increased in both dose groups depending on the duration of exposure. At the FT4 level, it was determined that there was no statistically significant difference in the comparison between the exposure duration.

1. Introduction

The increase in water pollution causes a serious threat to human and animal health (Rajper et al. 2018; Ullah et al. 2019; Nabi et al. 2019). This threat is mostly caused by industrial chemicals and oil. In terms of economic savings, the rapidly increasing water transportation in the world further increases this pollution. The penetration of toxic paint biocides into the aquatic environment over time, which is used against biological pollution, which is an important problem, especially in the shipping industry, brings an extra pollution load. Antifouling paints applied to the hulls of ships and other underwater structures to prevent the colonisation of microorganisms. In the past, tributyltin (TBT), an organotin compound, was widely applied as an antifouling agent in marine antifouling paints on ship hulls to control fouling organisms (Fent 1996). Recently, the use of TBT in marine antifouling paints has been strictly prohibited due to its persistence and toxic effects on non-target aquatic organisms. Since the global ban of TBT, metal pyrithiones such as copper pyrithione (CuPT), zinc pyrithione (ZnPT), sodium pyrithione (NaPT) are frequently used as biocide components of new antifouling paint products registered on the market (Okamura and Mieno 2006). Pyrithione (N-hydroxypyridine-2-thione), usually applied in zinc, copper or salt form, is a broad spectrum fungistatic and antimicrobial agent NaPT exists as a mixture of two tautomeric forms: (I) 1-hydroxy-2 (1H)-pyridine, sodium salt, and (II) 2-pyridinethio-1-oxide, sodium salt. NaPT is also used in the cosmetics and metal industries. NaPT is also the degradation product of other metal pyrithiones. NaPT is a broad-spectrum antimicrobial compound added to process fluids used as a preservative to prevent certain manufacturing materials from deteriorating through bacterial and/or fungal growth. Additionally, NaPT is used as a biocide in many areas (RED 1996).

It has been reported that many chemicals discharged into aquatic environments have a high ability to disrupt the hormone and enzyme systems in aquatic vertebrates.

Determination of acute toxicity tests of chemicals is crucial. Researchers reveal the toxic effect of chemicals on organisms with LC_{50}-96h and LC_{50}-24h acute tests, and determine the direction and severity of the possible toxic effect of the chemical. Accordingly, legal regulations can be made for chemicals (Rand and Petrocelli 1985).

Contaminants often cause relatively rapid changes in the haematological parameters of fish (Johansen et al. 1994; Rezania et al. 2018; Rizkalla et al. 2021). The hematological parameters are a common method used to monitor the response of fish to various toxic substances, reflecting the ecological status of the habitat, and to determine the lethal effects of the pollutant (Larsson et al. 1985; Malik et al. 2015). Therefore, blood parameters are used as an indicator of health prediction and show toxicological symptoms of organisms, especially fish (Singh 1995; Pimpão et al. 2007). To evaluate the toxic stress effect of the compound, many hematological parameters such as RBC count, WBC count, hemoglobin content, analysis of MCV, MHC and MCHC hematological indices are considered (Khattak and Hafeez 1996; Nagaraju et al. 2017).

It has also recently reported that endocrine organs can be used as biomarkers to determine the effect of various pollutants (Lawrence and Hemingway 2008). In fish, growth hormone (GH) participates in almost all important physiological processes in the body, including ionic and osmotic balance, lipid, protein and carbohydrate metabolism, skeletal and soft tissue growth, regulation of reproduction and immune function (Reinecke et al. 2005). Due to these roles of GH, it is essential to investigate the effect of aquatic environment pollution on the growth hormone of fish. Since adverse environmental conditions and various factors prevent the normal functioning of the body in vertebrates, homeostatic processes are insufficient to maintain physiological processes (R.F. and H. 2002; Todini et al. 2006). In this context, changes in the levels of hormones such as thyroid hormones (TH), adrenocorticotropic hormone (ACTH), glucocorticoids, catecholamines and prolactin are observed in the case of stress. The response to stress in an organism occurs as an increase in catecholamine (epinephrine, norepinephrine) levels as the primary response and an increase in glucocorticoids (cortisol), a secondary hormonal response due to the release of ACTH hormone (Wendelaar Bonga 1997; Mommsen et al. 1999).

The common carp (*Cyprinus carpio* L.) is an indicator fish of inland waters. Because of its high nutritional value and cheapness, it is frequently used in hunting and aquaculture. Therefore, in this study, common carp, which has a high tolerance to environmental effects, was preferred. Thus, it is aimed to better reveal the possible effects of NaPT.

For the reasons explained above, to determine the possible effects of NaPT in common carp, it was aimed to determine the toxicity of LC_{50}-24h and to investigate its effect on hematological and hormonal changes.

2. Materials And Methods

2.1. Fish and experimental conditions

The common carps used in the experiment have the weight of 60–70 g and a length of 15–17 cm. The study's Ethics Committee approval was issued by the Faculty of Veterinary, Selcuk University (letter No. 68429034/42, dated 16.12.2016). The fish used in the experiment were transported from Yedikir Fisheries Farm, which is run by the 73rd Branch Office of General Directorate of State Hydraulic Works, Ministry of Forest and Water Affairs, to the laboratory and were fed in 150-L glass aquariums (depending on the specific density of fish 10 g/L) for 20 days, and their adaptation to the environment was ensured. The experiment was carried out with a semi-static system in natural light (12h light-12h dark). During the experiment, fish were nourished with Pinar pellet feed (45% protein, 19% fat, crude fiber 3%) once a day. Twelve hours after the last feeding dose, hormone parameters analysis of the fish was done following the experiment.

The water used in the experiment was first passed through a central water treatment unit to fix its total hardness and pH, and from there, it was transferred to glass aquariums. The water temperature used in the experiment is 12 ± 0.1 ^{o}C, dissolved oxygen 7.9 ± 0.2 mg/L, pH 7.7 ± 0.1 and total hardness value 142.7 ± 1.64 mg/L.

2.2. Determination of LC50-24h of NaPT acute toxicity and its exposure

After the acclimation period, preliminary studies were conducted to determine the LC_{50} values of sodium pyrithione used in the experiment. Animals were not fed during the last 24 hours of acclimatisation and throughout the experiment. 10 fish were randomly placed in each glass aquarium with a capacity of 150 L. Trials were carried out at 10, 20, 40, 80, 160 and 200 µg/L dose concentrations of sodium pyrithione and the LC_{50}-24h value was determined. Mortality was evaluated 24, 48, 72 and 96 hours after the start of the tests The dead fish were immediately removed from the aquarium. Behavioural changes were followed closely. The NaPT (Merck-Sigma Aldrich product no 329061) used for the experiment was purchased from the commercial laboratory chemical materials company and stored in a refrigerator at + 4°C until the stock solution was prepared. The main studies started to determine the LC_{50} values from the information obtained in the first trials. Mortality rates were determined at appropriate dose intervals. Probit regression analysis was used in the statistical analysis of the data obtained in the experiment. Accordingly, the LC_{50}-24 h dose of NaPT for common carp was determined as 102.76 µg/L (Table 1).

Additionally, 60 healthy fish were randomly allocated into 6 exposure groups (control goup = 0% µg/L NaPT for 24 h and 96 h; NaPT-10 = 10% of the LC50-24 h dose of NaPT for 24 h and 96 h; NaPT-20 = 20% of the LC50-24 h dose of NaPT for 24 h and 96 h) to investigate the effects of NaPT exposure on hematological parameters and some hormones of the carps. The experiment was repeated every day to keep the dose concentration constant.

2.3. Hematological analysis

The blood sample was taken from the dorsal aorta to glass tubes for hematological and hormone analyzes by using the "tail-cutting method" after applying phenoxyethanol anaesthetic to the fish. After

blood samples were taken, euthanasia was performed by exsanguination method. Hematological analysis of fish was performed using commercial kits (Cat. No: WD1153) in Ms4 (Melet Schloesing, France) veterinary blood count device (Korkmaz and Orun 2020). In this context, RBC counts, Hb amount, Hct value, MCV, MCH, MCHC, WBC, lymphocyte, monocytes and granulocyte ratios were determined.

2.4. Serum hormone analysis

Hormonal analysis was performed in Animal Morphology and Physiology Research Laboratory at Aksaray University, Scientific and Technological Application and Research Center. The analysis of GH, IGF-1 and ACTH was measured by ELISA (Cusabio, China). DRG (USA) brand ELISA kits were used for the analyses of cortisol and thyroid hormones. In this context, serum growth hormone (GH) and insulin-like growth factor (IGF-1), stress hormones [adrenocorticotropic hormone (ACTH), cortisol] and thyroid gland hormones [serum thyroid stimulating hormone (TSH), free and total triiodothyronine (FT3-TT3) and thyroxine (FT4- TT4) hormones] analyzes were performed.

2.5. Statistical analysis

Statistical analysis was performed using the SPSS 24.0 statistical programme. All data are presented as arithmetic mean ± SD. One-way ANOVA followed by Duncan's multiple range tests used to analyse the experimental parameters. The Student-t test was used to determine the differences between acute and subchronic durations of the same dosage group. A value of $p < 0.05$ was considered statistically significant.

3. Results

The 24-hour acute LC_{50} value (95% confidence interval) of NaPT calculated for *Cyprinus carpio* using the static bioassay system was determined as 102.7643 µg/L (74.51–134.62) (Table 1). The control group mortality was zero.

Table 1
Acute toxicity of sodium pyrithione on common carp *Cyprinus carpio* L. (24 h LC_{50})

Point	Concentration (µg/L)	95% Confidence Limits	Slope ± SE
LC 1.00	54.38	12.68–74.83	8.42 ± 2.91
LC 5.00	65.5248	22.25–85.12	
LC10.00	72.3721	29.84–91.70	
LC15.00	77.3934	36.24–96.82	
LC50.00	102.7643	74.51-134.62	
LC85.00	136.4522	109.84-261.05	
LC90.00	145.9196	116.52-315.52	
LC95.00	161.1678	126.05-421.47	
LC99.00	194.1949	143.93-736.36	
Note: Control group (Theoretical Spontaneous Response Rate) = 0.0000			

A statistically significant decrease observed in the erythrocyte count (RBC), hemoglobin (Hb) amount, hematocrit (Hct) values, total leukocyte count (WBC), lymphocyte (%) and monocyte (%) ratios in the NaPT exposed group compared to the control group. However, increases in granulocyte (%) rates were found to be statistically significant ($p < 0.05$). Additionally, in the comparison of duration; the reductions in the ratios of RBC, Hb, Hct, WBC, lymphocytes and monocytes in the dose groups exposed to NaPT at 24h and 96h duration were found to be statistically significant ($p < 0.05$) (Table 2).

Table 2
Hematological parameters in common carp fish (*Cyprinus carpio* L. 1758) exposed to sodium pyrithione

Hematological parameters	Exposure duration (hour)	Experimental groups		
		Control	**NaPT-10**	**NaPT-20**
Red blood count (RBC) $mm^3/10^6$	24	1.75 ± 0.08^{a}	$1.35 \pm 0.04^{b*}$	$1.07 \pm 0.03^{c*}$
	96	1.76 ± 0.04^{a}	1.25 ± 0.03^{b}	1.03 ± 0.02^{c}
Hemoglobin (Hb) g/dL	24	12.7 ± 0.10^{a}	$9.8 \pm 0.12^{b*}$	$8.6 \pm 0.21^{c*}$
	96	12.5 ± 0.12^{s}	9.2 ± 0.14^{b}	8.2 ± 0.18^{c}
Hematocrit (Hct) %	24	35.7 ± 1.14^{a}	31.5 ± 1.15^{b}	$28.7 \pm 1.07^{c*}$
	96	35.6 ± 1.15^{a}	28.2 ± 1.20^{b}	24.9 ± 1.05^{c}
Total Leukocyte (WBC) $mm^3/10^3$	24	10.5 ± 0.62^{a}	10.4 ± 0.48^{a}	$9.1 \pm 0.54^{b*}$
	96	10.7 ± 0.58^{a}	10.5 ± 0.49^{a}	8.4 ± 0.42^{b}
Lymphocyte %	24	77.0 ± 1.12^{a}	71.0 ± 1.56^{b}	$62.5 \pm 1.72^{c*}$
	96	76.0 ± 1.05^{a}	68.7 ± 1.42^{b}	57.5 ± 1.45^{c}
Monocyte %	24	3.0 ± 0.14^{a}	2.7 ± 0.15^{a}	$2.3 \pm 0.16^{b*}$
	96	3.1 ± 0.15^{a}	2.6 ± 0.18^{a}	2.0 ± 0.18^{b}
Granulocyte %	24	20.0 ± 0.88^{c}	26.4 ± 0.70^{b}	35.2 ± 0.25^{a}
	96	19.9 ± 0.75^{c}	28.8 ± 0.72^{b}	$40.5 \pm 0.22^{a*}$

[a.b.c]: The averages shown by the different letters on each column are statistically different ($p < 0.05$, $n = 10$).

(*****): It shows the difference between the averages depending on the duration ($p < 0.05$).

The changes in serum GH and IGF-1 levels caused by NaPT exposure in the common carp fish shown in Fig. 1. The decreases in serum GH and IGF-1 levels of common carp fish exposed to NaPT for 24 h and 96 h in both dose groups compared to the control group were found to be statistically significant ($p < 0.05$). In the comparison between durations, the decrease in serum GH and IGF-1 levels between both dose groups was found to be statistically significant ($p < 0.05$).
Serum ACTH and cortisol hormone analysis results are shown in Fig. 2. Compared to the control group, the increase in serum ACTH and cortisol levels of common carp fish exposed to NaPT in both dose groups at 24 h and 96 h was statistically significant ($p < 0.05$). In the comparison between durations, the increase in serum ACTH and cortisol levels in both dose groups was found to be statistically significant ($p < 0.05$).

As a result of the analysis of serum thyroid hormones, a decrease in the serum TSH, FT3 and FT4 levels of common carp fish exposed to NaPT for 24 h and 96 h was found to be statistically significant in both dose groups compared to the control group, while an increase in serum TT3 and TT4 levels were found to be statistically significant for 24 h and 96 h in both dose groups ($p < 0.05$). In the comparison of exposure durations, the decrease in serum TSH, TT3 and TT4 levels was statistically significant between both dose groups ($p < 0.05$), while the decrease in serum FT3 levels between NaPT-20 dose groups was statistically significant ($p < 0.05$). There was not a statistically significant difference was found in serum FT4 levels in the comparison between durations ($p > 0.05$) (Table 3).

Table 3
Serum Thyroid-stimulating hormone (TSH), serum free triiodothyronine (FT3), serum free thyroxine (FT4), serum total triiodothyronine (TT3), and serum total thyroxine (TT4) levels in common carp (*Cyprinus carpio* L. 1758) exposed to sodium pyrithione

Groups	TSH (µLU/mL)		FT3 (pg/mL)		FT4 (ng/dL)		TT3 (ng/mL)		TT4 (nmol/mL)	
	24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h
Control	5.55 ± 0.09^{a}	5.55 ± 0.09^{a}	3.82 ± 0.04^{a}	3.90 ± 0.03^{a}	0.98 ± 0.06^{a}	1.04 ± 0.06^{a}	1.21 ± 0.12^{b}	1.24 ± 0.12^{b}	5.77 ± 0.24^{b}	5.77 ± 0.24^{b}
NaPT-10	4.90 ± 0.07^{b*}	4.55 ± 0.06^{b}	3.46 ± 0.08^{b}	3.51 ± 0.08^{b}	0.81 ± 0.08^{b}	0.82 ± 0.03^{b}	1.65 ± 0.12^{a*}	1.36 ± 0.10^{a}	8.05 ± 0.34^{a*}	7.55 ± 0.21^{a}
NaPT-20	4.69 ± 0.04^{b*}	4.04 ± 0.08^{b}	2.56 ± 0.05^{c*}	2.26 ± 0.11^{c}	0.71 ± 0.04^{c}	0.71 ± 0.02^{c}	1.75 ± 0.14^{a*}	1.38 ± 0.14^{a}	8.28 ± 0.23^{a*}	7.04 ± 0.17^{a}
[a.b.]: The averages shown by the different letters on each column are statistically different ($p<0.05$, $n=10$).										
(*): It shows the difference between the averages depending on the duration ($p<0.05$, $n=10$).										

4. Discussion

Pollutants released into the aquatic environment cause undesirable changes in water quality, and these chemicals, which causes pollution discharged into water resources, can pose significant hazards to the environment and health. One of these chemicals is sodium pyrithione (NaPT).

NaPT (N-hydroxypyridine-2-thione) is a broad-spectrum antimicrobial and fungistatic agent that is the active ingredient in certain anti-dandruff shampoos and are common additive inadhesives, sealants, aerosols, and marine antifouling paints. After it was determined that tributyltin (TBT), which is used as an antifouling, has a toxic effect for the aquatic environment and organisms, pyrithiones were started to be used as antifouling. Copper pyrithione (CuPT), zinc pyrithione (ZnPT), and sodium pyrithione (NaPT) are metallic pyrithions. Although there are many literature studies on the serious toxic effects of CuPT and Zn PT on organisms, there is limited information on the harm of NaPT on living organisms. In these limited studies, NaPT has been found to cause low toxicity in mammals. Although paralysis of the hind limbs is the most typical symptom of intoxication, this effect has not been reported in every experimental animal (Jung et al. 2019). Due to its high cytotoxicity, it has only been used in low concentration in *in vitro* genotoxicity tests so far.

It has been confirmed that ZnPT causes oxidative stress in both the gills and liver of *Gambusia holbrooki* and causes specific and irreversible tissue changes (Nunes et al. 2015). Marcheselli et al. (2011) determined that when the sea mussel (*Mytilus galloprovincialis*) is exposed to sublethal concentrations of ZnPT, adverse effects cause stress in in vivo, and stress causes severe DNA damage in the gills and gastrointestinal gland. They also demonstrated the levels of bioaccumulation after the biocide exposure.

Zhao et al. (2018) found that ZnPT has a broad spectrum of toxicity, causing growth retardation, tissue pathological and physiological changes in the heart, liver, kidney, eye, and bone in zebrafish. In another study, it was found that hindbrain ventricular morphogenesis did not expand as usual at the embryological level (Elsen et al. 2008).

Zhao et al. (2018), an obvious concentration-dependent delay of hatching rate result from ZnPT exposure was detected. Simultaneously, a significant defective development in elongation of the yolk sac and shortening of the entire body length was also observed. Exposure to ZnPT showed inhibitory effects on the pigmentation of zebrafish embryos, possibly due to its inhibitory potential on tyrosinase activity.

NaPT, a pyrithione, is an antimicrobial and antifungal agent widely used in the cosmetics, mining and fuel industries (Dinning et al. 1998). NaPT inhibits substrate transport processes in fungi and bacteria (Chandler and Segel 1978). NaPT is a substance that is easily administered, absorbed from the gastrointestinal tract and intact skin, with known toxicity (Mitoma et al. 1983). After oral administration in rats, rabbits and monkeys, NaPT rapidly absorbed from the gastrointestinal tract and absorption was 88%-100% after oral administration in rats. In studies with mice, 0.15–0.6% of radioactive labelled NaPT was detected in the liver and 0.4–0.8% in other organs after oral or intraperitoneal administration (Ziller 1977; Greim 2012). It has been reported that a single dose of dermal application of radioactive NAPT in rats, it is detected in the muscle and liver close to the application site (Parekh et al. 1970). Because it is cytotoxic, only low concentrations can be tested. In rats, mice, and rabbits given single or multiple doses

of NaPT, alternating hind limb paralysis has been reported as a typical manifestation of poisoning. Irreversible eye damage has been seen in species with *tapetum lucidum*. Since high concentrations are cytotoxic, the genotoxic effect of NaPT tested at low concentrations could not be demonstrated at high concentrations (Greim 2012).

Sublethal concentrations of chemicals can affect important physiological functions such as inhibition of growth, reproduction and biochemical events that may adversely affect the population of the species. Therefore, sublethal toxicity has increasing importance in ecotoxicity testing (Walker 2006).

In the data obtained in this study, mortality rates increased with increasing NaPT concentration and exposure duration. There are limited studies in the literature on the toxic effect of NaPT on fish. However, similar results have been reported in studies on the effects of different toxic substances on different aquatic organisms. Bao et al. (2012), in their study to investigate the toxicity of CuPT and ZnPT on *Elasmopus rapax*, found the 96 h median lethal concentration (LC_{50}) as 11.5 µg/L and 21.5 µg/L, respectively. Additionally, in a study with *Danio rerio* zebrafish, the acute toxicity values of ZnPT were found to be LC_{50} (95% CI) 96 h 0.073 µM (Zhao et al. 2018). Mohamat-Yusuff et al. (2018), investigating the toxicity of CuPT on Japanese medaka fish, found the LC_{50} 96 h value to be 16.58 mg/L. Mochida et al. (2006) found the LC50 for *Pagrus major*, a teleost for CuPT and ZnPT antifouling, to be 9.3 and 98.2 µg/L, respectively, and 2.5 and 120 µg/L for *Heptacarpus futilirostris*, a crustacean. Gümüş et al. (2015) found the LC_{50} 48 h value to be 7.32 µg/l for *Dreissena polymorpha* exposed to CuPT pyrithione.

The ecotoxicity of ZnPT to aquatic test organisms is *Lepomis macrochirus* 96 h LC_{50} 0.021 mg/l (Madsen et al. 2000), *Onchorhynchus mykiss* 96 h LC_{50} 0.0032 mg/l (Madsen et al. 2000), *Pimephales promelas* 96 h LC_{50} 0.0026 mg/l (Madsen et al. 2000), *Salvelinus fontinalis* 96 h LC_{50} 0.008 mg/l (Madsen et al. 2000), *Pagrus major* 96 h LC_{50} 0.098 mg/l (Onduka et al. 2010). Additionally, 48 and 72 h LC_{50} values for the ZnPT invasive species *Dreissena polymorpha* were found to be 51.9 and 11.5 µg/L, respectively (Yildirim et al., 2015).

In aquatic toxicology, an LC_{50} of less than 1000 ppb is considered a “very toxic” substance, a substance between 1000 and 10000 ppb is considered a “moderately toxic” substance, and a higher than 10000 ppb is considered a “less toxic” substance. In this study, the LC_{50} value of NaPT for common carp was determined as 102.7643 µg/L. Therefore, according to this assessment, NaPT is a highly toxic substance for common carp.

Hematological and hormonal parameters are frequently used to reveal the toxic effects of chemicals in a short time. However, studies on the effects of pyrithions such as NaPT, CuPT and ZnPT on hematological and hormonal parameters in fish are not very common in the literature. For this reason, the results of studies on the effects of pyrithiones on these parameters and the effects of other toxic chemicals on these parameters will be more meaningful in the evaluation of the results of this study.

It has been reported that *P. olivaceus* exposed to ZnPT (10 and/or 50 µg/L) has a decrease in RBC and WBC levels and no significant change in Hb level (Min et al. 2019). Vaiyanan et al. (2015) reported a decrease in Hb level because of the exposure of *Cyprinus carpio* to monocrotophos pesticide. It was determined that the exposure of *Labeo rohita* to cypermethrin at sublethal concentration showed a significant decrease in RBC count, Hb amount and hematocrit values compared to the control group (Adhikari et al. 2004). Similarly, Jee et al. (2005) reported that exposure of Korean rockfish (*Sebastes schlegelii*) to cypermethrin decreased RBC count, Hb level and Hct values. They concluded that these results might be due to the destructive effect of the toxic chemical on the cell membrane. They also suggested that the decrease in RBC count, Hb level and Hct level could cause erythrocyte hemolysis and/or irreparable scars and damage to gill morphology and function. It is also suggested that the decrease in Hb level may be due to the increase in the rate of destruction of hemoglobin or the decrease in the rate of synthesis. Decreases in RBC, Hb, and Hct, which are highly correlated with hematological parameters, have been linked to inhibition of erythropoiesis, red blood cell destruction, hematopoietic tissue destruction in kidney and spleen, and impaired hemopoietic process. A significant increase in the number of WBCs of *Cyprinus carpio* (Vaiyanan et al. 2015) exposed to monocrotophos and *Channa punctatus* (Jayaprakash and Shettu 2013) exposed to deltamethrin has been reported. The increase in WBC count has been evaluated as a response to the immune system due to toxic stress. Similar to the above studies, in the present study, NaPT caused decreases in RBC, Hb and HCT values, while it increased WBC count.

It has been determined that 4-nonylphenol reduces the serum IGF-1 level of Atlantic salmon and decreases somatic growth (Arsenault et al. 2004). It has been reported that pesticides induce growth retardation done studies with *Oreochromis niloticus*, *Chrysicthys nigrodigitatus*, *Clarias gariepinus* (Sweilum 2006; Hanson et al. 2007; Bose et al. 2011), *Danio rerio* (Cook et al. 2005) and *Oncorhynchus tshawytscha* (Baldwin et al. 2009). In this study, it is assumed that the reason for the decrease in GH and IGF-1 levels is the fact that many toxic substances such as heavy metals and pesticides may inhibit the hypothalamic-pituitary axis.

The results of the current study are similar to the observations of Ajani (2008), who noted that stress causes hormonal changes in fish and impairs its production, and Vijayavel et al.'s (2006) studies where they reported that stressful situation elicits neuroendocrine response in fish.

It has been reported that fish produce an adaptive response to stress by secreting HPI axis hormones (ACTH and cortisol) (Schreck, 1990) and adaptive responses in fish take a very long time (Alexander and Ingram 1992). In studies on most fish species, it has been reported that there is a high increase in plasma cortisol levels in a short time after stress (Barton 2002). It has been reported that cortisol has a significant effect on the dynamics of toxic substances in fish (Mommsen et al. 1999) and intended to meet the increased energy needs of animals when faced with stress (Barton 2002; Langiano and Martinez 2008). It has been reported in studies that fish increase the plasma glucose level as a very common response under stress conditions and this is aimed at meeting the increased energy demand of tissues such as the brain, gill and muscle (Barton 2002), and cortisol mediates the hyperglycemic response in many teleost

species (Wendelaar Bonga 1997). Studies have reported that the inhibition of ACh receptors affects the release of ACTH (Hontela 2005; Aluru and Vijayan 2006). This study also suggests that the increase in ACTH and cortisol levels activates the HPI axis adaptively to overcome stress by eliminating the neurotoxic effect.

Fish endocrine responses can also be considered as early warning indicators to assess the response to toxic stress and pollution (Hontela et al. 1993). Additionally, the measurement of circulating hormone levels can provide additional information on the lethal effects of chemicals (Folmar et al. 1993). Thyroid hormones (THs) are expressed in neuroendocrine activation of the hypothalamic-pituitary-thyroid axis (Eales 2006; Zoeller et al. 2007) and are active in almost all vertebrate cells (Heijlen et al. 2013). The thyroid gland is responsible for the secretion of thyroid hormones, which regulate growth, development and basal metabolism. It is also responsible for the secretion of calcitonin, which regulates calcium homeostasis. Many environmental pollutants negatively affect thyroid function and development (Li et al. 2008; He et al. 2012; Katuli et al. 2014; Naderi et al. 2014, 2015). Hypothalamic-pituitary-thyrotropin (HPT) hormones play an important role in growth metabolism in fish due to their effects on energy metabolism, lipid metabolism (Leatherland 1994; Lynshiang and Gupta 2000; Eales 2006; Blanton and Specker 2007) and genetic transcription (Zoeller et al. 2002; Li et al. 2009; Liu et al. 2011). Serum TSH, T3 and T4 levels are widely used as reliable indicators of thyroid function in experimental animals, and changes in serum concentrations of these hormones may reflect impaired synthesis and secretion in peripheral metabolism (Kelly 2000; Yousif and Ahmed 2009). Tagawa and Hirano (1991) reported that the partial reduction of T3 and T4 hormones in *Oryzias latipes* fish species has a significant effect on hatchability, survival and development of young fish. Additionally, studies have shown that the inhibition of the thyroid gland prevents metamorphosis in larvae and juvenile fish (Miwa and Inui 1987), while exogenous T3 and T4 administration causes early metamorphosis in fish (Brown 1997).

Similar to the results of this study, they reported increased TSH levels in *Liza aurata* (Oliveira et al. 2011) collected from contaminated areas, and *Danio rerio* exposed to triadimefon (Liu et al. 2011) and perchlorate (Patiño et al. 2003). Similar to this study, Yu et al. (2013) reported a significant decrease in T4 levels in zebrafish exposed to hexaconazole and tebuconazole fungicides. Also, Coimbra et al. (2005) stated that endosulfan exposure on Nile tilapia (*Oreochromis niloticus*) decreased T4 plasma level. It has been shown that monocrotophos (MCP), an organophosphate pesticide, decreased plasma TT3 levels and TT3-TT4 ratios in male goldfish (*Carassius auratus*) and had no effect on plasma TT4 levels (Zhang et al. 2013). It was also determined that exposure to malathion (10 and 20 ppm) and BHC (8 ppm) decreased plasma T4 level. It was also reported that fish exposed to BHC had a significant decrease in plasma T3 level (Yadav and Singh 1986). As with the results of the current study, other studies have also found that some pesticides reduce T3 and T4 activity levels in freshwater fish (Zhang et al. 2013; Khatun and Mahanta 2014; Ghelichpour et al. 2017; Nugegoda and Kibria 2017).

In this study and other studies, it has been reported that fish exposed to pesticides show a significant decrease in both TSH levels and T3 and T4 plasma levels compared to the control group. The increase and decrease in TSH can be explained by negative feedback arrangements (Wiersinga 2000). Because T4

decrease in plasma and/or lower TH production level by the pituitary, TSH increase can be expected (Patiño et al. 2003; Teles et al. 2005; Oliveira et al. 2011). Simultaneously, the decrease in plasma T3 level may have occurred due to the decrease in T4 synthesis or secretion (Li et al. 2008). Additionally, the decrease in plasma TT3 and TT4 levels because of increased exposure to NaPT may be due to possible changes in peripheral TH deiodination or metabolism because of the negative feedback mechanism. It has been shown that toxicological stress can affect the activity of 5'-deionidase (deio), which converts T4 to T3, by changing gene expression in fish (Wiersinga 2000; Wei et al. 2008; He et al. 2012). In the evaluations of the studies, it is stated that the decrease in the synthesis of TT3 and TT4, pesticides and pollution induces hyperplasia and hypertrophy of the follicular epithelium in the thyroid tissues, cause thyroid endocrine disruption, which causes an imbalance in T4 and T3 levels. Therefore, we hypothesise that NaPT may have had a cytotoxic effect on thyroid gland follicles. Simultaneously, anti-thyroid peroxidase antibodies against TSH receptors may be synthesised. Moreover, stimulated enzymatic metabolism of T3 in the liver may have reduced circulating TT3 levels (Zhang et al. 2014).

5. Conclusion

As a result of this study, it was concluded that sodium pyrithione, which is used as a broad-spectrum fungistatic and antimicrobial agent in the surface coating of ships, especially in the maritime sector, has a highly toxic effect on carp fish and has a negative hormonal effect. Also, the scarcity of studies on the toxicity of sodium pyrithione on fish makes it important to conduct more studies on this subject.

Declarations

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Author contribution This study was conceived and designed by N.K., K.E., G.N.Ö., B.E., M.I.D., A.D., H.P. and İ.Ö. All authors performed experimental work. K.E. and N.K. performed histopathological work, M.I.D and A.D. performed antioxidant enzyme analysis, İ.Ö. and G.N.Ö. performed hematological analysis, H.P. and B.E. performed hormones analysis. İ.Ö. performed statistical analysis. N.K. performed manuscript writing. N.K. and M.İ.D. performed English proofreading. All authors read and approved the final manuscript.

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Code availability Not applicable.

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Figures

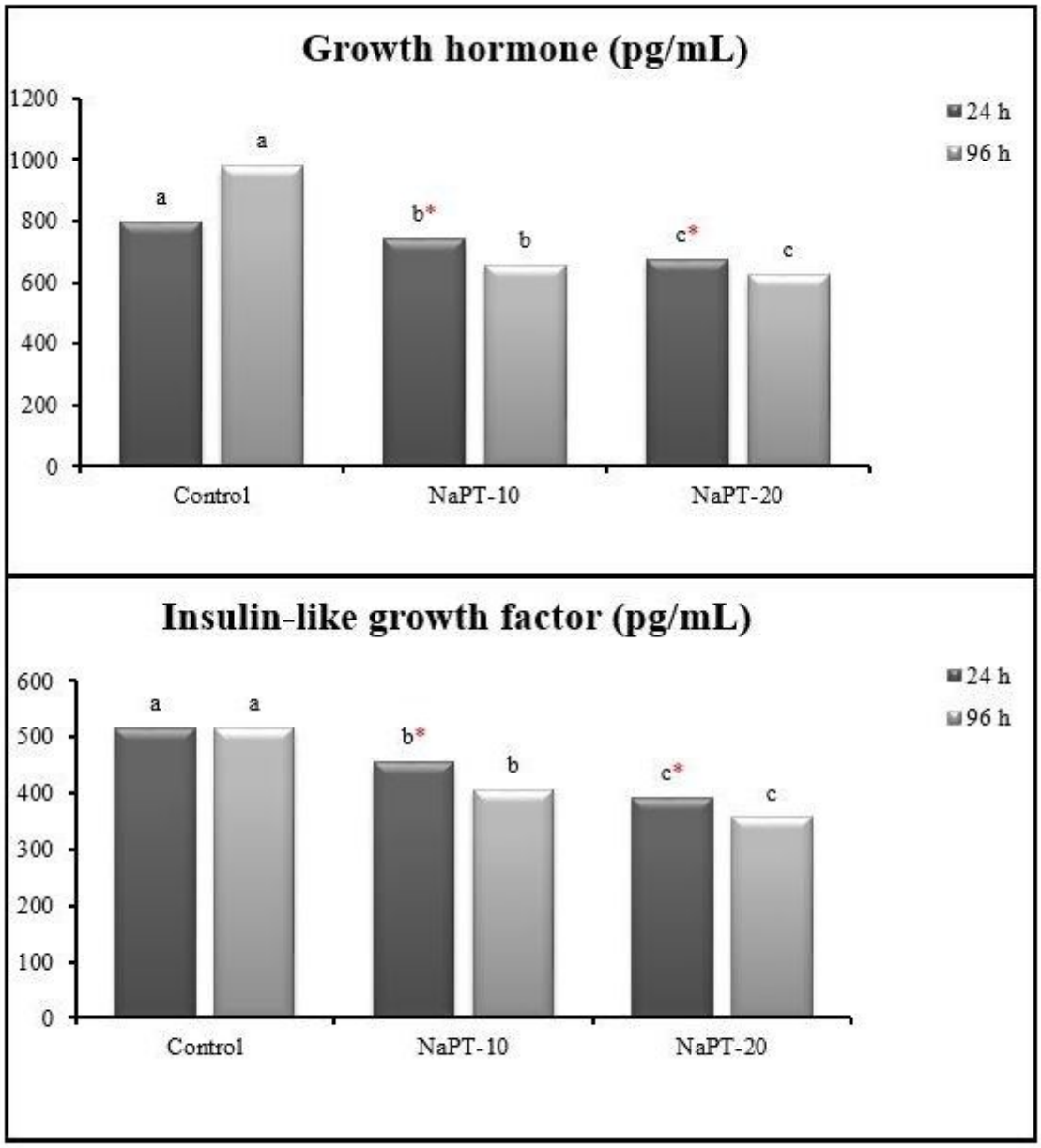

Figure 1

Serum growth hormone (GH) and serum insulin-like growth factor (IGF-1) levels in common carp fish (*Cyprinus carpio* L. 1758) exposed to sodium pyrithione

[a.b.c.]: The averages shown by the different letters are statistically different ($p<0.05$, $n=10$).

(*): It shows the difference between the averages depending on the duration ($p<0.05$, $n=10$).

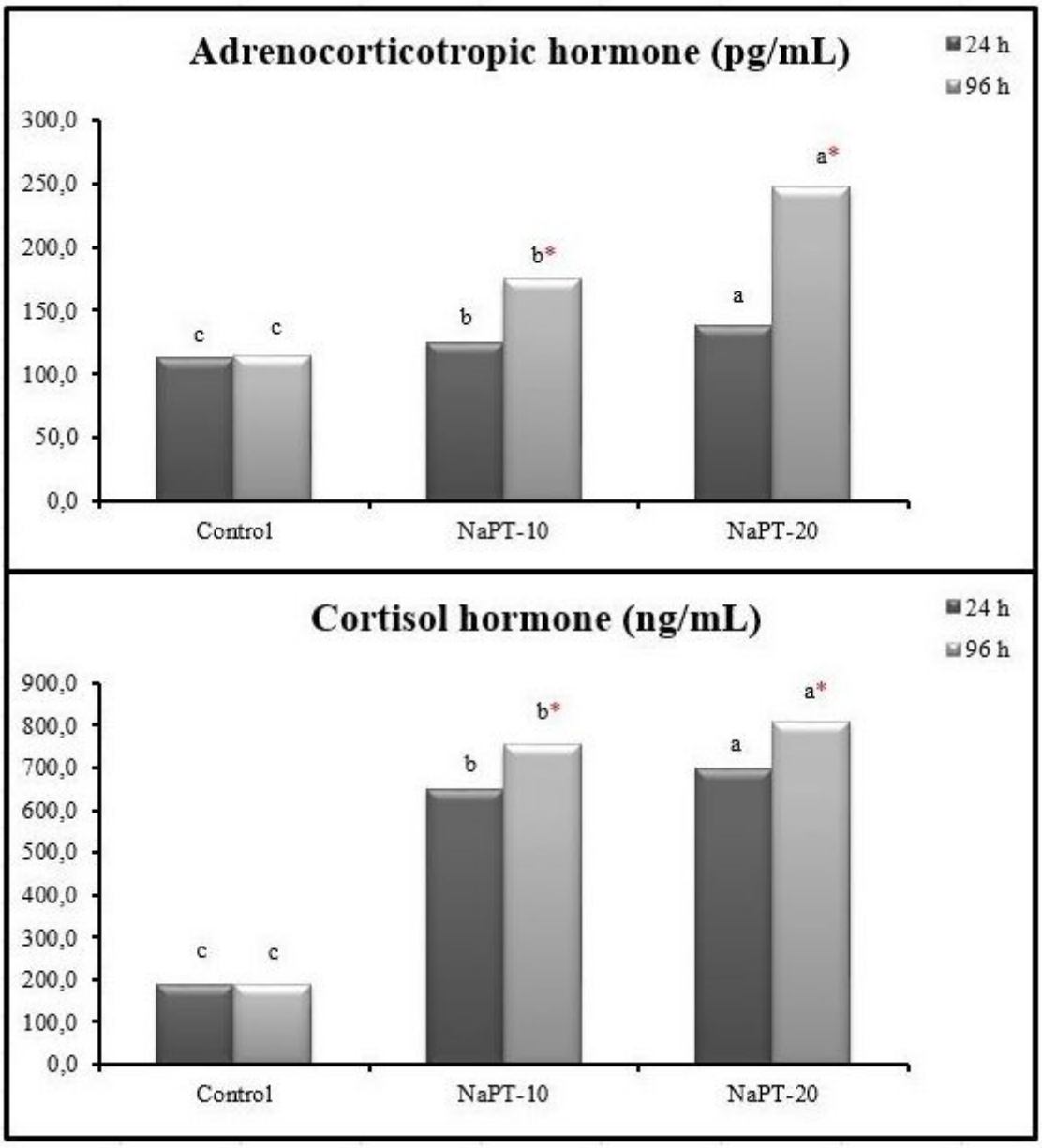

Figure 2

Serum adrenocorticotropic hormone (ACTH) and serum cortisol hormone levels in common carp (*Cyprinus carpio* L. 1758) exposed to sodium pyrithione

[a.b.c.]: The averages shown by the different letters are statistically different ($p<0.05$, $n=10$).

(*): It shows the difference between the averages depending on the duration ($p<0.05$, $n=10$).